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Pantoprazole, a proton pump inhibitor, increases orthodontic tooth movement in rats

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ABSTRACT

Objective(s): Pantoprazole, is a proton pump inhibitor (PPI) prescribed for the treatment of upper gastrointestinal disorders, which in high doses has been suggested to decrease calcium absorption leading to hypocalcaemia and therefore osteoporosis. The aim of this study was to assess whether pantoprazol, could alter the rate of orthodontic tooth movement (OTM) in rats.

Materials and Methods: A time course study was established using 72 rats which were divided into six groups of 12 samples each (four: vehicle; eight: pantoprazole + vehicle). Pantoprazole at a dose of 200 mg/kg suspended in carboxymethyl cellulose (0.25 percent) was administered by a gastric tube. The upper incisors and first molars were ligated by a 5 mm nickel-titanium closed-coil spring to deliver an initial force of 60 g. Animals were euthanized two weeks after orthodontic treatment followed by assessment of tooth movement and histomorphometric evaluation of the detached maxillae. Lateral skull radiographs were obtained once a week, starting from the first day to the 6th week of the study. OTM and bone density data were analyzed using independent sample t-test and repeated measures ANOVA.

Results: No significant changes in OTM measurements and optical density were observed in vehicle-receiving animals during the study (P=0.994). OTM was significantly increased after six weeks pantoprazole therapy which continued until the 7th week of the experiment (P=0.007). Optical density significantly increased in the pantoprazole-treated rats after six weeks.

Conclusion: Long term PPI therapy at high doses could lead to osteoporosis and enhanced OTM.

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Introduction

Proton pump inhibitors (PPIs) such as pantoprazole act by irreversibly binding to H+/K+ ATPase ion-exchanger or the proton pump located in the apical surface of acid secreting parietal cells in the stomach (1). PPIs are regarded as remarkably safe and effective agents for treatment of upper gastrointestinal disorders (2). Although these drugs are one of the most frequently prescribed medications, have recently raised concern that taking them, particularly at high doses, may result in decreased calcium absorption, hypocalcaemia, and therefore osteoporosis (3, 4). Two population-based epidemiological studies in Denmark and UK have supported this hypothesis (5, 6).

The alveolar bone surrounds dental tissues and is constantly undergoing remodeling which is considered to be the basis of the orthodontic tooth movement (OTM) (7, 8). Application of mechanical forces during orthodontic treatment induces modifications in the remodeling of the supporting alveolar bone, resulting in a predominance of bone resorption in the pressure side, and an advantage of bone formation in the tension side (7). Osteoclasts elicit bone resorption and are generated from their precursors following force application. Some of these precursor cells already exist in the periodontal ligament, but others are recruited to the pressure side within 2 to 3 days. Differentiation and polarization of osteoclasts follow, which ultimately

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Table1. Timetable for drug administrations in each study group

	Group 6	Group 5	Group 4	Group 3	Group 2	Group 1
Week 1	Panto/Veh BDE	Panto/Veh	Panto/Veh	Panto/Veh	Panto/Veh	Panto/Veh App insertion
Week 2	Panto/Veh BDE	Panto/Veh	Panto/Veh	Panto/Veh	Panto/Veh App insertion	Panto/Veh
Week 3	Panto/Veh BDE	Panto/Veh	Panto/Veh	Panto/Veh App insertion	Panto/Veh	OTM measurement
Week 4	Panto/Veh BDE	Panto/Veh	Panto/Veh App insertion	Panto/Veh	OTM measurement	
Week 5	Panto/Veh BDE	Panto/Veh App insertion	Panto/Veh	OTM measurement		
Week 6	Panto/Veh BDE App insertion	Panto/Veh	OTM measurement			
Week 7 End	Panto/Veh OTM measurement	OTM measurement				

OTM: Orthodontic tooth movement; App: Appliance; Panto/Veh: Pantoprazole/vehichle administration, BDE: Bone densitometry evaluation

lead to resorption of osseous tissue. Osteoblasts are responsible for bone formation and are derived from local precursor cells in the tension side, which differentiate into mature osteoblastic cells that produce osteoid. This is ensued by gradual mineralization and generation of bone (9). It has been shown that local and systemic factors are able to reactivate osteoclasts which dissolve the minerals through secretion of hydrogen ions and degrade the organic matrix by liberating proteolytic enzymes. We have previously reported the effect of renal insufficiency, hypothyroidism, and cholestasis on the rate of OTM; therefore it may be logical to suggest that any factor causing systematic alteration of bone metabolism such as hypocalcemia, can potentially increase the rate of OTM (10-13). Rat models have been used in various investigations and their result was compared and generalized to similar human events (14-16). This study was conducted to determine whether PPIs could alter the rate of orthodontic tooth movement in rats.

Materials and Methods

Study design

The present investigation was designed as a time-course study and our sample consisted of 72 male Sprague-Dawley rats (nine weeks old) weighing from 200 to 250 g, which were divided into six groups of 12 animals each. Their diet consisted of soft laboratory food in order to facilitate eating after placement of the appliances and to reduce the possibility of the animals displacing their orthodontic devices. The humidity and temperature of the caging rooms were controlled and maintained with a 12 hr light and dark cycle. All experiment conditions and procedures strictly adhered to the guidelines described by the US National Institute of Health (NIH publication no. 85.23, revised 1985 for the Care and Use of Laboratory Animals and the study protocol were approved by the ethics committee of our university. Each of the six study groups were further divided into two subgroups including eight test animals that received pantoprazole 200 mg/kg in 0.25 percent carboxymethyl cellulose (CMC) by a gastric tube (pantoprazole treated rats) and four rats that received only CMC (vehicle only treated rats) (17). A 2-week course of orthodontic treatment was performed for all animals, during which the rats were given their routine experimental agents (pantoprazole or vehicle). The six groups were as follows: 1) a group that received pantoprazol and appliance from the first day of the study, 2) a group that received pantoprazole one week before the appliance insertion, 3) a group that received pantoprazole two weeks before the appliance insertion, 4) a group that received pantoprazole three weeks before the appliance insertion, 5) a group that received pantoprazole four weeks before the appliance insertion, 6) a group that received pantoprazole five weeks before the appliance insertion. Table1 shows the time table for drug administration, appliance insertion, radiography, and date of sacrifice in each study group.

Drug administration

Pantoprazole (Byc Gulden, Konstanz, Germany) at a dose of 200 mg/kg daily was dissolved in 0.25 percent sodium carboxymethyl cellulose (9004-32-4, Sigma-Aldrich, Saint Louis, MO, USA) and was administered via a fine gastric tube. In addition, 65 mg/kg intraperitoneal pentobarbital was used for general anesthesia and they were sacrificed by a 100 mg/kg pentobarbital overdose at the end of the study.

Survival study and general toxicity

Assessment of mortality and general toxicity including edema, cachexia, alopecia, and mortality were carried out daily in all groups while the body weight was measured twice a week through the whole experiment period.

Placement of orthodontic appliances

The orthodontic appliance design used in the present investigation was similar to that employed

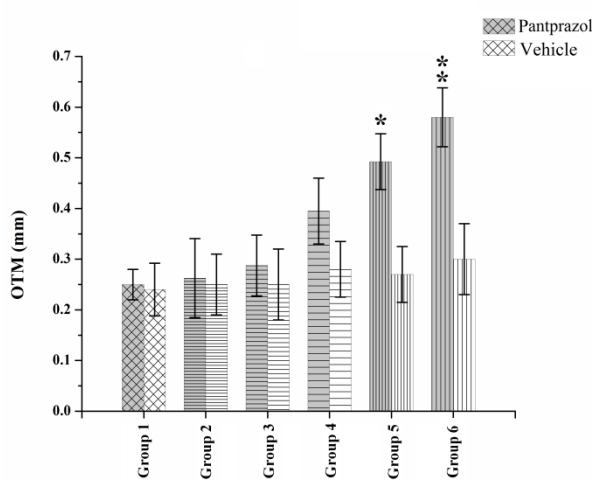

Figure 1. OTM measurements in six study groups: each group consisted of four rats comprising the vehicle-only group (receiving only vehicle) and eight rats constituting the pantoprazole-treated group who received pantoprazole + vehicle (*P<0.05; ** P<0.01)

by Shirazi *et al* (10). Briefly, nickel-titanium closed coil springs (NiTi, 3M Unitek, Monrovia, Calif, Hi-tek, 0.006 × 0.022 inch) were cut at a length of 5 mm and placed between the upper right first molar and incisors of all treated and vehicle-only animals to provide an initial force of 60 g. Reactivation was not performed during the 2-week study period due to the "flat load-deflection curve" of these appliances (12, 13). Maximum anchorage in the anterior region was achieved through uniting the right and left incisors with composite resin, therefore warranting minimal distal movement of the right incisor.

Measurement of tooth movement

Animals were sacrificed 2 weeks after orthodontic treatment by intraperitoneal administration of 100 mg/kg sodium pentobarbital. After removal of the maxillae, special trays covering the right three molars were fabricated of acrylic (Acropars, Iran) and impressions were made using polisyloxane impression material (Spidex, Colthene, Swiss) followed by pouring with dental stone type IV (SH 074, Ernst Hinrichs GmbH, Germany). Measurements of the mesiodistal spaces between the distal surface of the first- and the mesial aspect of the second-molar were performed on the stone casts, using a standard millimeter inter-proximal gauge (10, 11). Ten days before drug administration all animals were controlled regarding their healthiness. The baseline OTM in 8 normal rats with no drug or vehicle administration (data not shown) was also measured.

Bone densitometry evaluation

After a stable anesthesia with a dose of 65 mg/kg pentobarbital, a custom-made cephalostat was utilized to obtain lateral skull radiographs. These radiographs were obtained on occlusal fast films once a week (only in group 6), from the first to the 6th week of the study. Radiographic conditions including exposure (10 mA at 50 kV), film-tube distance (50 cm), and film types were identical for all animals. Bone density was assessed at the lower border of the mandible, exactly beneath the first molar, employing optical densitometry (12, 18). Baseline optical density of 8 rats (without receiving any drug or vehicle) was measured 10 days before the main study (data not shown).

Histological evaluation

To assess possible histologic changes due to drug administration and orthodontic treatment, the right hemimaxillae of all animals were surgically removed subsequent to sacrifice, and placed in ten percent formalin for 10 days. The specimens were decalcified in 5% formic acid for at least seven days, after washing with tap water. Routine sample processing was carried out and five micrometer mesiodistal sections were cut from paraffin blocks followed by hematoxylin and eosin staining of every fifth sections. The mesial roots of the first molars from the cementoenamel junction CEJ to the terminal point of the apex were analyzed on the slide with the largest root area. The microscopic observations were recorded for each case using an Olympus BX51 light microscope equipped with a digital camera (DP25, Olympus) and analysis software (DP2-BSW, Olympus). Osteoclasts were characterized as multinucleated cells with vacuolated eosinophilic to light basophilic cytoplasm, resided in Howship lacunae, and demonstrated ruffled borders when active. Osteoblastic activity was assessed by observation of surfaces with active bone formation surrounded by osteoid and plump basophilic, cuboidal osteoblasts. Measurements of periodontal ligament (PDL) width was performed in the widest area between the root- and bone-surfaces. Root resorption was determined by counting the number of resorptive lacunae, measurement of depth, and largest width of each of the craters. All parameters were recorded for both mesial and apical root aspects.

Statistical analysis

The results are reported as mean ± SE. Statistical analysis of body weight changes and OTM data were performed by independent sample t-test while repeated measures of ANOVA was used for evaluation of optical densitometry. Group differences were calculated by *post-hoc* analysis using LSD test. For all the tests, differences with values of $P < 0.05$ were considered significant.

Results

Survival study and general toxicity

After 5 weeks of the study, 2 rats died in the group receiving pantoprazole for 5 weeks before the appliance insertion. Administration of pantoprazol at the used dosage did not induce any general toxicity including edema, cachexia, and

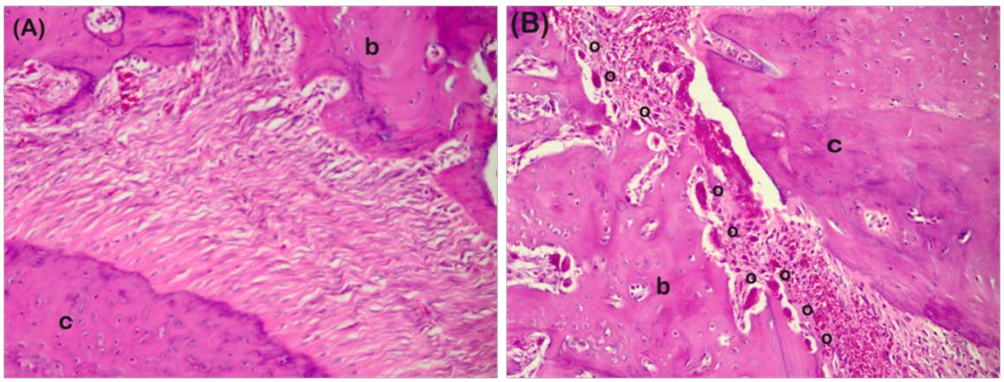

Figure 2. Microscopic view showing the periodontium (c: cementum; b: bone) of the mesial root in (A) a vehicle only rat with a low osteoclast count (hematoxylin and eosin staining; original magnification, ×400) and (B) an animal belonging to group F with a considerable number of osteoclasts (o) on the bone surface (original magnification, ×400)

alopecia. No significant difference in weight gain or loss was found between the pantoprazole treated and vehicle-only groups (P<0.05).

OTM findings

A significant change in OTM was not observed in the vehicle only groups during the experiment period (P=0.994). There was no significant difference between the vehicle only and pantoprazol treated rats until the 5th week of the investigation (P=0.067); however OTM differences became significant from the 6th week (P=0.005) and increased during the 7th week of the experiment (P=0.007). In other words, significant changes in tooth movement were found only in groups 5 and 6; but not in groups 1, 2, 3, and 4 (Figure 1).

Bone densitometry findings

Mandible optical density at the studied location (beneath the first molar) did not change in the vehicle-only rats during the study period (P=0.873), while it increased from the 4th to 6th week of drug administration in the pantoprazole treated animals leading to a significant difference (P=0.037) at week 6 in group 6.

Histopathological findings

A minimum of three slides was prepared for each sample which included three molars and their supporting tissues. Various numbers of osteoclasts were observed in 15 pantoprazole treated rats which were mainly localized to the mesial and apical surfaces of the mesial first molar roots. The lowest osteoclast number was counted in one of the vehicle only rats, while the highest was seen in one of the animals in group 6 (Figure 2). This number was roughly equal between groups 1, 2, and 3, but higher in group 4 compared with the three previous ones. Group 5 demonstrated more osteoclasts than group 4, but a lower number in comparison to group 6 which received pantoprazole for 5 weeks before orthodontic treatment. Two pantoprazole treated rats demonstrated a large number of osteoclasts in the distal aspects of the studied root, one (group 6) of which also showed hypercementosis and a hypercellular PDL with considerable inflammation. In the other sample that had received pantoprazole for two weeks and two weeks orthodontic treatment, tissue hemorrhage was observed in the PDL. Hypercementosis was found in three pantoprazole treated rats from groups 3, 5, and 6; with the former exhibiting a substantial number of cementoblasts. Prominent osteoblastic activity in the bony surface adjacent to the mesial surface of the mesial root existed in 09/2008 two pantoprazole treated rats (groups 4 and 6); however increased osteoblastic numbers were seen in the distal aspect of several other specimens. Only one animal in group 4 showed three particles of calcified material in the pulp chamber. Seven pantoprazole treated rats had resorptive areas on the mesial and/or distal surfaces of the mesial root, ranging in size from 0.014 mm to 0.4174 mm in width and from 0.003 mm to 0.1185 mm in depth. The distribution and size of the resorptive defects were roughly similar between the pantoprazole and vehicle-only groups. The width of the PDL was measured in the mesial and apical portions of the root; ranged from 0.0816 mm to 0.8000 mm. The smallest (apex: 0.0816 mm; mesial: 0.0968 mm) and largest (apex: 0.8000 mm; mesial: 0.4738 mm) PDLs were observed in groups 2 and 6, respectively.

Discussion

The objective of the present study was to investigate if long-term administration of PPIs can increase orthodontic tooth movement. According to our results, rats treated with pantoprazol at a dose of 200 mg/kg for 7 weeks had no sign of general toxicity including weight loss, edema, cachexia, and alopecia. This confirms the idea that PPIs are remarkably safe and have a wide therapeutic window. OTM did not significantly change in the vehicle-only rats during our study which indicates that animal age may not significantly affect orthodontic tooth movement and perhaps

osteoporosis. Since there were no differences between body weight changes of the vehicle-only and pantoprazole treated animals during the investigation, it might be possible that the body weight in itself, did not affect OTM. Orthodontic tooth movement significantly increased after six weeks of pantoprazole treatment and this upward trend continued to the last week of the experiment. This implies that the effects of pantoprazole on OTM could be time-dependent. Radiographic assessment of the animals employed in the current investigation showed that optical density had increased significantly after the 6th week of drug administration, suggesting that mandible bone radiographic studies beneath the first molar are precise enough to show bone density alterations.

Considering the limited number of cases with histologic evaluation and the possible technical problems inherent to tissue processing, it may not be possible to form a definitive opinion on the events that take place in osteoporosis at the cellular level. However, the pattern of distribution in osteoclast numbers showed a slight increase from the vehicle-only rats to the animals receiving pantoprazole for 5 weeks and orthodontic treatment for 2 weeks, which seems to be in line with our OTM and findings and densitometric analysis. It is noteworthy that statistical analysis was not performed on the microscopic data and therefore exact interpretation would not be rational. Further studies with biomarkers reflecting both number and function of osteoclasts (e.g. CTX-I ± TRAP 5b) is suggested to clarify cell changes in osteoporosis.

In several studies, we have previously reported that some mechanisms which may interfere with calcium homeostasis can influence OTM; these include vitamin D deficiency, cholestasis which causes vitamin D deficiency, levothyroxine administration that reduces serum calcium level and bone density, and chronic renal failure which is one of the situations that alters calcium metabolism in bone tissue (11, 12, 19). A considerable number of investigations have reported that PPIs significantly increase the risk of hip fractures, particularly in those receiving long-term and high-doses of this agent (3, 20, 21). Roux *et al* (22) observed an increased risk of vertebral fractures in postmenopausal women that take omeprazole. Previous research findings showed a significant relationship between chronic use of PPIs and hip fracture in a dose dependent manner, which led to the proposition that acid suppressive therapy, might weaken calcium absorption and therefore enhance the risk of osteoporosis (3, 4, 20, 21). For example a randomized crossover trial has previously demonstrated that omeprazole therapy can significantly decrease calcium absorption from calcium carbonate when ingested by elderly women (23). In addition, Cui *et al* (24) reported that long-term omeprazole administration to young male rats suppresses bone mineralization. The underlying mechanisms responsible for the increased risk of hip fracture and bone defects are not fully understood. However, considering the fact that long-term and high-dose PPI therapy reduces gastric acid secretion and since disintegration and dissolution of calcium salts is pH-dependant, it may be hypothesized that disintegration and dissolution slows down with increasing pH, possibly causing impairment in calcium absorption, which ultimately leads to osteopenia and consequently increased OTM (23). Further studies should be performed to confirm our findings and determine the mechanism of the association between PPIs use and risk of increasing OTM. Considering the increasing demand for orthodontic treatment among adults (25), some of whom may be receiving pantoprazole and the prevailing application of PPI in children and teenagers (26), modification of treatment modalities in patients using this drug may be necessary in clinical settings.

Conclusion

In summary, the data presented here imply that OTM was significantly enhanced after six weeks of pantoprazole therapy with a dose of 200 mg/kg and this increase continued until the 7th week of drug administration. A significant increase in optical density was detected in pantoprazole-treated animals after 6 weeks of treatment. Histopathological findings confirm the results obtained from the OTM measurements and densitometric analysis performed in this investigation. Our findings show that long term PPI therapy at high doses increases OTM in rats.

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